East Search
6-22-04

L Number	Hits	Search Text	DB	Time stamp
1	857	melanotransferrin or p97 or (membrane adj bound adj trasnferrin adj like)	USPAT; US-PGPUB; EPO	2004/06/22 14:27
2	369	(melanotransferrin or p97 or (membrane adj bound adj trasnferrin adj like)) and @py<=2001	USPAT; US-PGPUB; EPO	2004/06/22 14:27
3	6	((melanotransferrin or p97 or (membrane adj bound adj trasnferrin adj like)) and @py<=2001) and GPI	USPAT; US-PGPUB; EPO	2004/06/22 14:28
4	188	(melanotransferrin or p97 or (membrane adj bound adj trasnferrin adj like)) and (cartilage or chondrocyte$ or chondro$)	USPAT; US-PGPUB; EPO	2004/06/22 14:29
5	41	((melanotransferrin or p97 or (membrane adj bound adj trasnferrin adj like)) and @py<=2001) and (cartilage or chondrocyte$ or chondro$)	USPAT; US-PGPUB; EPO	2004/06/22 14:29
6	46	(((melanotransferrin or p97 or (membrane adj bound adj trasnferrin adj like)) and @py<=2001) and GPI) or (((melanotransferrin or p97 or (membrane adj bound adj trasnferrin adj like)) and @py<=2001) and (cartilage or chondrocyte$ or chondro$))	USPAT; US-PGPUB; EPO	2004/06/22 14:29

	Document ID	Issue Date	Title	Inventor	Current OR
1	US 20010034042 A1	20011025	Complexes of peptide-binding fragments of heat shock proteins and their use as immunotherapeutic agents	Srivastava, Pramod K.	435/68.1
2	US 6331396 B1	20011218	Arrays for identifying agents which mimic or inhibit the activity of interferons	Silverman, Robert H. et al.	435/6
3	US 6322790 B1	20011127	Compositions and methods for eliciting an immune response using heat shock/stress protein-peptide complexes in combination with adoptive immunotherapy	Srivastava, Pramod K.	424/193.1
4	US 6310034 B1	20011030	Agouti polypeptide compositions	Woychik, Richard P. et al.	514/2
5	US 6261535 B1	20010717	Diagnostic methods for targeting the vasculature of solid tumors	Thorpe, Philip E. et al.	424/1.49

	Document ID	Issue Date	Title	Inventor	Current OR
6	US 6258360 B1	20010710	Prodrugs activated by targeted catalytic proteins	von Borstel, Reid et al.	424/182.1
7	US 6218166 B1	20010417	Adjuvant incorporation into antigen carrying cells: compositions and methods	Ravindranath, Mepur H. et al.	435/366
8	US 6187312 B1	20010213	Compositions and methods using complexes of heat shock protein 90 and antigenic molecules for the treatment and prevention of infectious diseases	Srivastava, Pramod K.	424/193.1
9	US 6162436 A	20001219	Compositions and methods using complexes of heat shock protein 90 and antigenic molecules for the treatment and prevention of neoplastic diseases	Srivastava, Pramod K.	424/193.1

	Document ID	Issue Date	Title	Inventor	Current OR
10	US 6156302 A	20001205	Adoptive immunotherapy using macrophages sensitized with heat shock protein-epitope complexes	Srivastava, Pramod K.	424/93.1
11	US 6143299 A	20001107	Compositions and methods using complexes of heat shock protein gp96 and antigenic molecules for the treatment and prevention of infectious diseases	Srivastava, Pramod K.	424/193.1
12	US 6139841 A	20001031	Compositions and methods using complexes of heat shock protein 70 and antigenic molecules for the treatment and prevention of infectious diseases	Srivastava, Pramod K.	424/193.1
13	US 6136315 A	20001024	Compositions and methods using complexes of heat shock protein 70 and antigenic molecules for the treatment and prevention of neoplastic diseases	Srivastava, Pramod K.	424/193.1

	Document ID	Issue Date	Title	Inventor	Current OR
14	US 6111081 A	20000829	Lactoferrin variants and uses thereof	Conneely, Orla M. et al.	530/400
15	US 6093399 A	20000725	Methods and compositions for the specific coagulation of vasculature	Thorpe, Philip E. et al.	424/182.1
16	US 6083602 A	20000704	Incontinent garments	Caldwell, J. Michael et al.	428/77
17	US 6080399 A	20000627	Vaccine adjuvants for immunotherapy of melanoma	Gajewski, Thomas F. et al.	424/85.2
18	US 6060064 A	20000509	Chimeric virus-like particle antigen presentation and delivery system	Adams, Sally Elizabeth et al.	424/199.1

	Document ID	Issue Date	Title	Inventor	Current OR
19	US 6051230 A	20000418	Compositions for targeting the vasculature of solid tumors	Thorpe, Philip E. et al.	424/178.1
20	US 6040251 A	20000321	Garments of barrier webs	Caldwell, J. Michael	442/123
21	US 6036955 A	20000314	Kits and methods for the specific coagulation of vasculature	Thorpe, Philip E. et al.	424/136.1

	Document ID	Issue Date	Title	Inventor	Current OR
22	US 6030618 A	20000229	Therapeutic and prophylactic methods using heat shock proteins	Srivastava, Pramod K.	424/184.1
23	US 6017540 A	20000125	Prevention and treatment of primary and metastatic neoplastic diseases and infectious diseases with heat shock/stress protein-peptide complexes	Srivastava, Pramod K. et al.	424/193.1
24	US 6004555 A	19991221	Methods for the specific coagulation of vasculature	Thorpe, Philip E. et al.	424/181.1
25	US 6004554 A	19991221	Methods for targeting the vasculature of solid tumors	Thorpe, Philip E. et al.	424/178.1

	Document ID	Issue Date	Title	Inventor	Current OR
26	US 5993800 A	19991130	Methods for prolonging the expression of a heterologous gene of interest using soluble CTLA4 molecules and an antiCD40 ligand	Linsley, Peter S. et al.	424/93.21
27	US 5985270 A	19991116	Adoptive immunotherapy using macrophages sensitized with heat shock protein-epitope complexes	Srivastava, Pramod K.	424/93.71
28	US 5981194 A	19991109	Use of p97 and iron binding proteins as diagnostic and therapeutic agents	Jefferies, Wilfred A. et al.	435/7.1
29	US 5965132 A	19991012	Methods and compositions for targeting the vasculature of solid tumors	Thorpe, Philip E. et al.	424/1.49
30	US 5935576 A	19990810	Compositions and methods for the treatment and prevention of neoplastic diseases using heat shock proteins complexed with exogenous antigens	Srivastava, Pramod K.	424/184.1
31	US 5925568 A	19990720	Release and mobilization of haematopoietic cells	Comer, Michael Berisford et al.	435/378
32	US 5912116 A	19990615	Methods of measuring analytes with barrier webs	Caldwell, J. Michael	435/5
33	US 5877289 A	19990302	Tissue factor compositions and ligands for the specific coagulation of vasculature	Thorpe, Philip E. et al.	530/387.1

	Document ID	Issue Date	Title	Inventor	Current OR
34	US 5874164 A	19990223	Barrier webs having bioactive surfaces	Caldwell, J. Michael	428/306.6
35	US 5863538 A	19990126	Compositions for targeting the vasculature of solid tumors	Thorpe, Philip E. et al.	424/136.1
36	US 5856245 A	19990105	Articles of barrier webs	Caldwell, J. Michael et al.	442/76
37	US 5855866 A	19990105	Methods for treating the vasculature of solid tumors	Thorpe, Philip E. et al.	424/1.49

	Document ID	Issue Date	Title	Inventor	Current OR
38	US 5840502 A	19981124	Methods for enriching specific cell-types by density gradient centrifugation	Van Vlasselaer, Peter	435/7.21
39	US 5837251 A	19981117	Compositions and methods using complexes of heat shock proteins and antigenic molecules for the treatment and prevention of neoplastic diseases	Srivastava, Pramod K.	424/193.1
40	US 5830464 A	19981103	Compositions and methods for the treatment and growth inhibition of cancer using heat shock/stress protein-peptide complexes in combination with adoptive immunotherapy	Srivastava, Pramod K.	424/93.71
41	US 5807703 A	19980915	Secreted proteins and polynucleotides encoding them	Jacobs, Kenneth et al.	435/69.1
42	US 5776427 A	19980707	Methods for targeting the vasculature of solid tumors	Thorpe, Philip E. et al.	424/1.49

	Document ID	Issue Date	Title	Inventor	Current OR
43	US 5660827 A	19970826	Antibodies that bind to endoglin	Thorpe, Philip E. et al.	424/152.1
44	US 5612185 A	19970318	Method for identifying tumor cells in cell cycle arrest	Uhr, Jonathan W. et al.	435/7.23
45	EP 1018343 A1	20000712	Use of p97 and iron binding proteins as diagnostic and therapeutic agents	FOOD, MICHAEL et al.	
46	WO 9401463 A1	19940120	USE OF p97 AND IRON BINDING PROTEINS AS DIAGNOSTIC AND THERAPEUTIC AGENTS	JEFFERIES, WILFRED A et al.	

East Search 6-22-04

L Number	Hits	Search Text	DB	Time stamp
1	17	kato-yukio.in. and (p97 or melanotransferrin or chondro$ or cartilage)	USPAT; US-PGPUB; EPO	2004/06/22 14:57

	Document ID	Issue Date	Title	Inventor	Current OR
1	US 20030009020 A1	20030109	Gene originating in human chondrocyte	Kato, Yukio et al.	536/23.2
2	US 20020169301 A1	20021114	Novel bHLH type transcription factor genes DEC2	Fujimoto, Katsumi et al.	536/23.1
3	US 6392022 B1	20020521	Gene originating in human chondrocyte	Kato, Yukio et al.	536/23.1
4	US 5670338 A	19970923	DNA encoding bone morphogenetic proteins, host cells transformed there by, and uses thereof	Murakami, Kazuo et al.	435/69.1
5	US 5607920 A	19970304	Concanavalin a binding proteins and a 76kD chondrocyte membrane protein (CMP) from chondrocytes and methods for obtaining same	Kato, Yukio et al.	514/13
6	US 5453419 A	19950926	Xenopus laevis bone morphogenetic proteins and use thereof	Murakami, Kazuo et al.	514/12
7	EP 1219303 A1	20020703	CHONDROGENESIS PROMOTERS	KATO, YUKIO et al.	
8	EP 1215283 A1	20020619	NOVEL bHLH TYPE TRANSCRIPTION FACTOR GENE DEC2	FUJIMOTO, KATSUMI et al.	
9	EP 1120651 A1	20010801	METHOD AND REAGENT FOR ASSAYING ARTHRITIS-ASSOCIATED MELANOTRANSFERRIN	KATO, YUKIO et al.	
10	EP 1033403 A1	20000906	GENE ORIGINATING IN HUMAN FETAL CHONDROCYTES	KATO, YUKIO et al.	
11	EP 1020517 A1	20000719	GENE ORIGINATING IN HUMAN CHONDROCYTE	KATO, YUKIO et al.	
12	WO 9928458 A1	19990610	GENE ORIGINATING IN HUMAN FETAL CHONDROCYTES	KATO, YUKIO et al.	
13	WO 9902677 A1	19990121	GENE ORIGINATING IN HUMAN CHONDROCYTE	KATO, YUKIO et al.	
14	EP 811383 A1	19971210	REMEDIES FOR ARTHROSIS DEFORMANS AND INFLAMMATORY JOINT DISEASES	KATO, YUKIO et al.	
15	WO 9625944 A1	19960829	REMEDIES FOR ARTHROSIS DEFORMANS AND INFLAMMATORY JOINT DISEASES	KATO, YUKIO et al.	
16	EP 635518 A1	19950125	New chondrocyte protein.	KATO, YUKIO et al.	

	Document ID	Issue Date	Title	Inventor	Current OR
17	EP 416578 A2	19910313	Protein, DNA and use thereof.	MURAKAMI, KAZUO et al.	

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FILE 'HOME' ENTERED AT 13:23:55 ON 22 JUN 2004

=> FIL MEDLINE SCISEARCH EMBASE BIOSIS
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.21       0.21

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=> s (melanotransferrin or p97) and GPI and py<=2001
   2 FILES SEARCHED...
L1          35 (MELANOTRANSFERRIN OR P97) AND GPI AND PY<=2001

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2           11 DUP REM L1 (24 DUPLICATES REMOVED)

=> d l2 1-11 ti au ab

L2   ANSWER 1 OF 11  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI   Use of p97 and iron binding proteins as diagnostic and
     therapeutic agents.
AU   Jefferies, Wilfred A. [Inventor, Reprint author]; McGeer, Patrick L.
     [Inventor]; Rothenberger, Sylvi [Inventor]; Food, Michael R. [Inventor];
     Yamada, Tatsuo [Inventor]; Kennard, Malcolm [Inventor]
AB   The invention related to a GPI-anchored p97 and a soluble form of p97 and
     derivatives thereof and methods for preparing the same.  Methods of using p97
     in modulating iron transport, in the delivery of therapeutic agents, and in
     the treatment of conditions involving disturbances in iron metabolism are
     described.  The treatment and diagnosis of Alzheimer's Disease in view of the
     finding that p97 and transferrin receptor are markers for microglial cells
     associated with senile plaques are also described.


L2   ANSWER 2 OF 11     MEDLINE on STN                    DUPLICATE 1
TI   Differences in the expression and localization of human
     melanotransferrin in lepidopteran and dipteran insect cell lines.
AU   Hegedus D D; Pfeifer T A; Theilmann D A; Kennard M L; Gabathuler R;
     Jefferies W A; Grigliatti T A
AB   The ability of several lepidopteran and dipteran insect cell lines to express
     human melanotransferrin (p97), a glycosyl phosphatidylinositol (GPI)-anchored,
     iron-binding sialoglycoprotein, was assessed.  Spodoptera frugiperda-derived
     (Sf9) cell lines, transformed with the p97 gene under control of a baculovirus
     immediate-early promoter, were able to constitutively express the protein and
     correctly attach it to the outer cell membrane via a GPI anchor as
     demonstrated by PI-PLC treatment.  In contrast, stable constitutive expression
     could not be demonstrated with cell lines derived from either Drosophila
     melanogaster (Kc1 or SL2) or Lymantria dispar (Ld652Y) despite the observation
     that p97 could be detected in transient expression assays.  This may indicate
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that the long-term expression and accumulation of **p97** is inhibitory to Drosophila cells, possibly due to improper localization of the protein and resultant competition for cellular iron. In stably transformed Sf9 cells, **p97** was expressed on the cell at a maximal level of 0.18 microg/10(6) cells and was secreted at a maximal rate of 9.03 ng/10(6) cells/h. This level was comparable to the amount expressed with the baculovirus system (0.37 microg/10(6) cells and 31.2 ng/10(6) cells/h) and transformed CHO cells (0.88 microg/10(6) cells and 7.8 ng/10(6) cells/h). Deletion of the **GPI** cleavage/attachment site resulted in an eightfold increase in the secretion rate of **p97**, when compared to the intact construct suggesting that the rate-limiting step involves processing of the **GPI** anchor.

L2 ANSWER 3 OF 11 MEDLINE on STN DUPLICATE 2
TI Characterization of the human leukocyte **GPI**-anchored glycoprotein CDw108 and its relation to other similar molecules.
AU Angelisova P; Drbal K; Cerny J; Hilgert I; Horejsi V
AB The CDw108 glycoprotein is expressed on the surface of some leukemic cell lines, erythrocytes and on activated lymphocytes. Its surface expression is rapidly upregulated following various activating stimuli (PHA, PWM, Con A, PMA, anti-CD3) and subsequently gradually decreases. The molecule is anchored in the membrane via glycosylphosphatidylinositol (**GPI**) moiety, it has molecular mass of 75-80 kDa and pI of 5.0-5.5. Endoglycosidase F and H reduce its apparent size as determined by SDS PAGE by approx. 15 and 22 kDa, respectively. It is a component of large, detergent-resistant **GPI**-complexes associated with protein kinases. In addition to the previously described identity of CDw108 with the JMH blood group antigen, we demonstrate here its identity to the previously described glycoprotein recognized by monoclonal antibodies H105 and KS.2, and exclude its identity with another **GPI**-anchored glycoprotein of similar size, **melanotransferrin** (gp97).

L2 ANSWER 4 OF 11 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 3
TI Increasing **GPI**-anchored protein harvest concentrations from suspension and porous microcarrier CHO cell cultures
AU Sunderji R; Piret J M; Kennard M L (Reprint)
AB Chinese hamster ovary (CHO) cells expressing the human melanoma tumour antigen, **p97**, were used to develop a controlled release process for the production of recombinant glycosyl-phosphatidylinositol (**GPI**) anchored proteins. The cells were cultured either in suspension or immobilized on porous microcarriers and **p97** was selectively cleaved from the cell surface by the bacterial enzyme, phosphatidylinositol-phospholipase C (PI-PLC). The kinetics of **p97** cleavage from the cell surface by PI-PLC was shown to be approximated by Michaelis-Menten kinetics. The recovered **p97** concentrations were increased by reusing the PI-PLC enzyme solution to harvest multiple batches of cells. A convenient PI-PLC assay was developed to monitor the harvesting process and to determine the stability of PI-PLC under harvesting conditions. Although the PI-PLC was stable under harvesting conditions, it rapidly adsorbed to the cell surface and was depleted from the reused enzyme solution. In order to maintain PI-PLC activity, it was necessary to add fresh PI-PLC to the reused enzyme solution before harvesting a fresh batch of cells. The maximum **p97** concentration that could be obtained from harvesting CHO cells cultured on porous microcarriers was limited by the dilution effects of sample removal, adding fresh PI-PLC and liquid associated with settled microcarriers. A model was developed that adequately predicted the **p97** concentration after each harvest and the maximum **p97** concentration that could be achieved by this harvesting method. The dilution effects were minimized by harvesting from centrifuged

suspension culture cells and the harvested **p97** concentration was increased by over sixfold to 0.64 mg/mL. (C) 1997 John Wiley & Sons, Inc.

L2 ANSWER 5 OF 11 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 4
TI Expression of cell surface **GPI**-anchored human **p97** in baculovirus-infected insect cells
AU Kennard M L; Shimizu K Y; Gabathuler R; Rothenberger S; Theilmann D; Jefferies W A (Reprint)
AB The baculovirus/insect cell system (Autographa californica multiple nuclear polyhedrosis virus/Spodoptera frugiperda Sf9 cells) was used to express the **GPI**-anchored human melanomatumor antigen, **melanotransferrin** or **p97**. This system served to study the expression and productivity of recombinant **GPI**-anchored **p97** by insect cells. The Sf9 cells expressed a cell surface **GPI**-anchored form of **p97** as well as a soluble form of **p97** that did not appear to be derived from the **GPI** -anchored form of **p97**; Both recombinant forms, although Endo H resistant, migrated slightly faster (similar to 88 kDa) than the native **p97** (similar to 95-97 kDa). The insect **GPI**-anchored **p97** was sensitive to PI-PLC, which exposed a detectable crossreacting determinant. The Sf9 cell surface **p97** expression was similar to that of human melanoma (SK-MEL-28) cells, whereas the Sf9 cell specific secretion rate was 10-fold higher. Also Sf9 cells retained considerably higher levels of **p97** within the cell. The Sf9 cell surface expression of **p97** varied with time after infection, with the maximum expression, which appeared independent of multiplicities of infection greater than 1, occurring at 48 h. After 48 h, levels of cell surface and secreted **p97** fell whereas **p97** retained within the cell increased, which possibly reflected the lytic nature of the expression system. The successful expression of **GPI**-anchored human **p97** by the baculovirus/insect cell system not only provides a source of **p97** for further research but also is the basis of an alternative method for the commercial production of **GPI**-anchored proteins. (C) 1997 John Wiley & Sons, Inc.

L2 ANSWER 6 OF 11 MEDLINE on STN DUPLICATE 5
TI Pumping iron in the '90s.
AU Jefferies W A; Gabathuler R; Rothenberger S; Food M; Kennard M L
AB The role o f iron in cell division, cell death and human disease has recently gained increased attention. The best studied process for iron uptake into mammalian cells involves traps ferrin and its receptor. This review discusses evidence supporting the existence of other routes by which iron can enter mammalian cells. Specifically, iron uptake by the cell-surface **GPI**-linked traps ferrin homologue, melanotrans ferrin or **p97**, is described and possible functions of this traps ferrin-independent pathway are proposed.

L2 ANSWER 7 OF 11 MEDLINE on STN DUPLICATE 6
TI Coincident expression and distribution of **melanotransferrin** and transferrin receptor in human brain capillary endothelium.
AU Rothenberger S; Food M R; Gabathuler R; Kennard M L; Yamada T; Yasuhara O; McGeer P L; Jefferies W A
AB One method of iron transport across the blood brain barrier (BBB) involves the transferrin receptor (TR), which is localized to the specialized brain capillary endothelium. The **melanotransferrin** (MTf) molecule, also called **p97**, has been widely described as a melanoma specific molecule, however, its expression in brain tissues has not been addressed. MTf has a high level of sequence homology to transferrin (Tf) and lactoferrin, but is unusual because it predominantly occurs as a membrane bound, glycosylphosphatidylinositol (**GPI**) anchored molecule, but can also occur as a soluble form. We have recently demonstrated that **GPI**-anchored MTf provides a novel route for

cellular iron uptake which is independent of Tf and its receptor. Here we consider whether MTf may have a role in the transport of iron across the BBB. The distributions of MTf, Tf and the TR were studied immunohistochemically in human brain tissues. The distributions of MTf and TR were remarkably similar, and quite different from that of Tf. In all brain tissues examined, MTf and the TR were highly localized to capillary endothelium, while Tf itself was mainly localized to glial cells. These data suggest that MTf may play a role in iron transport within the human brain.

L2 ANSWER 8 OF 11 MEDLINE on STN DUPLICATE 7
TI A novel iron uptake mechanism mediated by **GPI**-anchored human **p97**.
AU Kennard M L; Richardson D R; Gabathuler R; Ponka P; Jefferies W A
AB The established process for iron uptake into mammalian cells involves transferrin and its receptor. Here, the role of the glycosyl-phosphatidylinositol (**GPI**)-linked transferrin homologue, **melanotransferrin** or **p97**, was studied using CHO cell lines defective in the transferrin receptor (TR) and transfected with human TR and/or human **p97**. The presence of **p97** doubled the iron uptake, which could be explained by the binding of one atom of iron to one molecule of **p97**. The internalization of iron was shown to be temperature sensitive and saturated at a media iron concentration of 2.5 micrograms/ml with a Vmax of 0.1 pmol Fe/10(6) cell/min and a Km of 2.58 microM for **p97**. Treatment of the cells with either phosphatidylinositol-phospholipase C or monoclonal antibodies against **p97** resulted in over a 50% reduction and a 47% increase in the iron uptake respectively. These data identify **p97** as a unique cell surface **GPI**-anchored, iron binding protein involved in the transferrin-independent uptake of iron in mammals.

L2 ANSWER 9 OF 11 MEDLINE on STN DUPLICATE 8
TI A transferrin-like **GPI**-linked iron-binding protein in detergent-insoluble noncaveolar microdomains at the apical surface of fetal intestinal epithelial cells.
AU Danielsen E M; van Deurs B
AB A **GPI**-anchored 80-kD protein was found to be the major component of detergent-insoluble complexes, prepared from fetal porcine small intestine, constituting about 25% of the total amount of protein. An antibody was raised to the 80-kD protein, and by immunogold electron microscopy of ultracryosections of mucosal tissue, the protein was localized to the apical surface of the enterocytes, whereas it was absent from the basolateral plasma membrane. Interestingly, it was mainly found in patches of flat or invaginated apical membrane domains rather than at the surface of microvilli. Caveolae were not found in association with these labeled microdomains. In addition, the 80-kD protein was seen in apical endocytic vacuoles and in tubulo-vesicular structures, suggesting that the apical microdomains are involved in endocytosis of the 80-kD protein. By its NH2-terminal amino acid sequence, iron-binding capacity and partial immunological cross-reactivity with serum transferrin, the 80-kD protein was shown to belong to the transferrin family, and it is probably homologous to **melanotransferrin**, a human melanoma-associated antigen. The 80-kD iron-binding protein was fully detergent-soluble immediately after synthesis and only became insoluble after gaining resistance to endo H, supporting a mechanism for exocytic delivery to the apical cell surface by way of detergent-insoluble glycolipid "rafts" that fuse with the plasmalemma at restricted sites devoid of microvilli.

L2 ANSWER 10 OF 11 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 9
TI GLYCOLIPID MEMBRANE-ANCHORED RECOMBINANT PROTEIN-PRODUCTION FROM CHO CELLS CULTURED ON POROUS MICROCARRIERS

AU KENNARD M L; PIRET J M (Reprint)

AB Recombinant proteins were harvested from Chinese hamster ovary (CHO) cells by a controlled release process, which increased the purity and concentration of the harvested protein. Recombinant human **melanotransferrin** (**p97**) was expressed linked to the outer surface of CHO cells by a glycosyl-phosphatidylinositol (**GPI**) membrane anchor. Cells were grown to confluence in T-flask cultures, and the **p97** harvested by replacing the growth medium for 30 min with phosphate-buffered saline (PBS) containing 10 mU/mL phosphatidylinositol- phospholipase C (PI-PLC). The **GPI** anchor was selectively cleaved by PI-PLC. In fresh medium, the CHO cells regained over 95% of their **p97** expression within 40 h. The process was repeated for eight harvests. Harvested protein concentrations varied from 1.5 to 3.8 mu g/mL due to difficulties in maintaining stable confluent T-flask cultures. Harvesting from cells growing on porous microcarriers was investigated to increase **p97** product concentrations and to overcome culture stability problems. Semicontinuous cultures were maintained in spinners for up to 76 days with average bioreactor cell densities of over 10(7) cell/mt. The **p97** was harvested:at up to 100 mu g/mL and 30% purity with protein production remaining stable for 14 harvest cycles. Production of high levels of **p97** from CHO cells was maintained at 0.5% serum. (C) 1994 John Wiley and Sons, Inc.

L2 ANSWER 11 OF 11 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 10

TI CONTROLLED-RELEASE PROCESS TO RECOVER HETEROLOGOUS GLYCOSYLPHOSPHATIDYLINOSITOL MEMBRANE-ANCHORED PROTEINS FROM CHO CELLS

AU KENNARD M L; FOOD M R; JEFFERIES W A; PIRET J M (Reprint)

AB A semicontinuous process has been developed to recover heterologous proteins at increased concentrations and purities. Proteins attached to mammalian cell membranes by glycosylphosphatidylinositol (**GPI**) anchors can be selectively released into the supernatant by the enzyme phosphatidylinositol-phospholipase C (PI-PLC). Chinese hamster ovary (CHO) cells, genetically engineered to express the **GPI** anchored, human melanoma antigen (**p97**), were used as a model system. These cells were grown in protein containing growth medium. During a brief harvesting phase the medium was replaced by phosphate buffered saline (PBS) containing 10 mU/mL of PI-PLC and the **GPI** anchored protein was cleaved from the cell surface and recovered in soluble form at up to 30% purity. After harvesting, the cells were returned to growth medium where the protein was re-expressed within 40 h. The growth rate, viability, and protein production of cells, repeatedly harvested over a 44-day period, were not adversely affected. This continuous cyclic harvesting process allowed recovery of a heterologous protein at high purity and concentrations and could be applied to the recovery of other **GPI** anchored proteins and genetically engineered **GPI** anchored fusion proteins. (C) 1993 John Wiley & Sons, Inc.

=> s (melanotransferrin or p97) and (chondrocyte or chondrogenesis or chondrogenic or cartilage)

L3 9 (MELANOTRANSFERRIN OR P97) AND (CHONDROCYTE OR CHONDROGENESIS OR CHONDROGENIC OR CARTILAGE)

=> dup rem l3

PROCESSING COMPLETED FOR L3

L4 6 DUP REM L3 (3 DUPLICATES REMOVED)

=> d l4 1-6 ti au ab

L4 ANSWER 1 OF 6 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
TI Anti-membrane-bound transferrin-like protein antibodies induce cell-shape change and **chondrocyte** differentiation in the presence or absence of concanavalin A
AU Oda R; Suardita K; Fujimoto K; Pan H; Yan W Q; Shimazu A; Shintani H; Kato Y (Reprint)
AB Membrane-bound transferrin-like protein (MTf), a glycosylphosphatidylinositol-anchored protein, is expressed at high levels in many tumors and in several fetal and adult tissues including **cartilage** and the intestine, as well as in the amyloid plaques of Alzheimer's disease, although its role remains unknown. MTf is one of the major concanavalin A-binding proteins of the cell surface. In this study, we examined the effects of anti-MTf antibodies and concanavalin A on cell shape and gene expression, using cultures of **chondrocytes** and MTf-overexpressing ATDC5 and C3H10T1/2 cells. In cultures expressing MTf at high levels, concanavalin A induced cell-shape changes from fibroblastic to spherical cells, whereas no cell-shape changes were observed with wild-type ATDC5 or C3H10T1/2 cells expressing MTf at very low levels. The cell-shape changes were associated with enhanced proteoglycan synthesis and expression of **cartilage** -characteristic genes, including aggrecan and type H collagen. Some anti-MTf antibodies mimicked this action of concanavalin A, whereas other antibodies blocked the lectin action. The findings suggest that the crosslinking of MU changes the cell shape and induces **chondrogenic** differentiation. MTf represents the first identification of a plant lectin receptor involved in cell-shape changes and the differentiation of animal cells.

L4 ANSWER 2 OF 6 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
TI Effects of overexpression of membrane-bound transferrin-like protein (MTf) on **chondrogenic** differentiation in vitro
AU Suardita K; Fujimoto K; Oda R; Shimazu A; Miyazaki K; Kawamoto T; Kato Y (Reprint)
AB Membrane-bound transferrin-like protein (MTf) is expressed in parallel with the expression of **cartilage**-characteristic genes during differentiation of **chondrocytes**, and the MTf level is much higher in **cartilage** than in other tissues. To investigate the role of MTf in **cartilage**, we examined the effects of growth factors on MTf expression in mouse prechondrogenic ATDC5 cells and the effect of MTf overexpression on differentiation of ATDC5 and mouse pluripotent mesenchymal C3H10T1/2 cells. In ATDC5 cultures, bone morphogenetic protein-2 and transforming growth factor-P as well as insulin induced MTf mRNA expression when these peptides induced **chondrogenic** differentiation. Forced expression of rabbit MTf in ATDC5 cells induced aggrecan, type II collagen, matrilin-1, type X collagen mRNAs, and cell-shape changes from fibroblastic cells to spherical **chondrocytes.** Accordingly, the synthesis and accumulation of proteoglycans were higher in MTf-expressing cultures than in control cultures. These effects of MTf overexpression correlated with the MTf protein level on the cell surface and decreased in the presence of anti-MTf antibody. However, the aggrecan mRNA level in the ATDC5 cells overexpressing MTf was lower than that in wild type ATDC5 cells exposed to 10 mug/ml insulin. MTf overexpression in C3H10T1/2 cells also induced aggrecan and/or type II collagen mRNA but not the spherical phenotype. These findings suggest that the expression of MTf on the cell surface facilitates the differentiation of prechondrogenic cells, although MTf overexpression alone seems to be insufficient to commit pluripotent mesenchymal cells to the **chondrocyte** lineage.

L4 ANSWER 3 OF 6 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
TI The human melanoma associated protein **melanotransferrin** promotes endothelial cell migration and angiogenesis in vivo
AU Sala R; Jefferies W A; Walker B; Yang J; Tiong J; Law S K A; Carlevaro M E; Di Marco E; Vacca A; Cancedda R; Cancedda F D; Ribatti D (Reprint)
AB **Melanotransferrin** is a member of the transferrin family, which is comprised of serum transferrin, lactoferrin and ovotransferrin, and is highly expressed on melanoma cells compared to normal melanocytes. Since melanoma is an higly vascularized tumour that expresses **melanotransferrin** at high levels, we tested purified recombinant **melanotransferrin** for its capability to induce angiogenesis in the chick chorioallantoic membrane. Macroscopic and microscopic evaluation of the vascular density demonstrated that **melanotransferrin** exerts an angiogenic response quantitatively similar to that elicited by fibroblast growth factor-2. Overexpression of vascular endothelial growth factor-receptor-2 was observed in newly formed vessels, suggesting that the angiogenic activity of **melanotransferrin** may depend on activation of endogenous vascular endothelial growth factor. In addition, when antibodies against vascular endothelial growth factor were included in the assay, the angiogenic response was inhibited by 50%. In a Boyden chamber assay purified recombinant **melanotransferrin** induced chemotactic migration of vascular cells, which was decreased in the the presence of anti-vascular endothelial growth factor antibodies suggesting an involvement of vascular endothelial growth factor present in endothelial cells also in this assay. However, **melanotransferrin** was found not to directly bind to integrin alpha(y)beta(3) or the vascular endothelial growth factor-receptor-2 as assessed in a BIAcore assay. A possible correlation between vascularization occurring during melanoma progression and the expression of **melanotransferrin** and vascular endothelial growth factor was established by immunolocalization of the two factors in sections of melanoma at different clinical steps of melanoma progression. These latter data strongly imply that **melanotransferrin** may participate in the vascularization of solid tumours and that inhibition of **melanotransferrin** could form the basis for intervention in tumours which use this pathway.

L4 ANSWER 4 OF 6 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
TI Structure and promoter analysis of the mouse membrane-bound transferrin-like protein (MTf) gene
AU Nakamasu K; Kawamoto T; Yoshida E; Noshiro M; Matsuda Y; Kato Y (Reprint)
AB Recently, we purified membrane-bound transferrin-like protein (MTf) from the plasma membrane of rabbit **chondrocytes** and showed that the expression levels of MTf protein and mRNA were much higher in **cartilage** than in other tissues [Kawamoto T, Pan, H., Yan, W., Ishida, H., Usui, E., Oda, R., Nakamasu, K., Noshiro, M., Kawashima-Ohya, Y., Fujii, M., Shintani, H., Okada, Y. & Kato, Y. (1998) Eur. J. Biochem. 256, 503-509]. In this study, we isolated the MTf gene from a constructed mouse genomic library. The mouse MTf gene was encoded by a single-copy gene spanning approximate to 26 kb and consisting of 16 exons. The transcription-initiation site was located 157 bp upstream from the translation-start codon, and a TATA box was not found in the 5' flanking region. The mouse MTf gene was mapped on the B3 band of chromosome 16 by fluorescence in situ hybridization. Using primary **chondrocytes**, SK-MEL-28 (melanoma cell line), ATDC5 (**chondrogenic** cell line) and NIH3T3 (fibroblast cell line) cells, we carried out transient expression studies on various lengths of the 5' flanking region of the MTf gene fused to the luciferase reporter gene. Luciferase activity in SK-MEL-28 cells was higher than in primary **chondrocytes.** Although no luciferase activity was detectable in NIH3T3 cells, it was higher in **chondrocytes** than in ATDC5 **chondrogenic** cells. These findings were

consistent with the levels of expression of MTf mRNA in these cells cultured under similar conditions. The patterns of increase and decrease in the luciferase activity in **chondrocytes** transfected with various 5' deleted constructs of the MTf promoter were similar to that in ATDC5 cells, but differed from that in SK-MEL-28 cells. The findings obtained with primary **chondrocytes** suggest that the regions between -693 and -444 and between -1635 and -1213 contain positive and negative cis-acting elements, respectively. The **chondrocyte** -specific expression of the MTf gene could be regulated via these regulatory elements in the promoter region.

L4 ANSWER 5 OF 6 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

TI Membrane-bound transferrin-like protein (MTf): structure, evolution and selective expression during **chondrogenic** differentiation of mouse embryonic cells

AU Nakamasu K; Kawamoto T; Shen M; Gotoh O; Teramoto M; Noshiro M; Kato Y (Reprint)

AB Mouse membrane-bound transferrin-like protein (MTf) cDNA was cloned to examine its expression during **chondrogenic** differentiation in the mouse embryonic cell line ATDC5, and to analyze the phylogenetic relationships among the MTfs of four animal species and 23 other transferrin members. Phylogenetic analysis indicated that the MTf gene diverged from the common ancestor gene earlier than the genes of the other transferrins such as serum transferrin, lactoferrin and ovotransferrin, and that the divergence occurred after the divergence of vertebrates and invertebrates. MTf, as well as the other transferrins, consists of two repeated domains. The similarity between the N-terminal and the C-terminal domains of MTf is much higher than that of the other transferrins, although the five amino acid residues required for iron binding were not conserved in the C-terminal domain of MTf in contrast to the conservation of these residues in both domains of the other transferrins. Among various adult mouse tissues, MTf mRNA was expressed at the highest level in **cartilage** and at a moderate level in the testis. MTf mRNA was expressed only at very low levels in the brain, spleen, thymus, muscle, lung, skin and intestine, and hardly detected in the heart, kidney, stomach and liver. In cultures of the mouse ATDC5 cell line, MTf is developmentally expressed in parallel with the expression of type IT collagen and aggrecan, in the pattern commensurate with the onset of **chondrogenesis** to form **cartilage** nodules. The structural characteristics and the expression pattern suggest that during development and in adult tissues, MTf has some functions that are different from those of other transferrins.

L4 ANSWER 6 OF 6 MEDLINE on STN DUPLICATE 1

TI Expression of membrane-bound transferrin-like protein **p97** on the cell surface of **chondrocytes.**

AU Kawamoto T; Pan H; Yan W; Ishida H; Usui E; Oda R; Nakamasu K; Noshiro M; Kawashima-Ohya Y; Fujii M; Shintani H; Okada Y; Kato Y

AB A concanavalin-A-binding protein of 76 kDa was purified from the plasma membrane fraction of rabbit **chondrocyte** cultures. Amino acid sequencing of the N-terminal region and of tryptic peptides of the protein, in addition to sequencing of its cDNA revealed that this protein is highly similar to the tumour-associated antigen **p97.** Hence, it was concluded that this protein is the rabbit form of **p97.** Western blotting, Northern blotting and reverse-transcription PCR analyses indicated that rabbit **p97** is expressed at high levels in **cartilage** and **chondrocytes**, but is barely detectable in the bone, liver, kidney, small intestine, eye, pancreas, heart, testis, skeletal muscle, spleen and fibroblasts. Immunocytochemical and immunohistochemical analyses demonstrated that **p97** is expressed in the plasma membrane of **chondrocytes. p97** transcript was detected in all zones of the **cartilage** but the level was

relatively low in the hypertrophic zone. These findings suggest that p97 is involved in maintaining the cell surface characteristics of **chondrocytes.**

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     2004
L1            35 S (MELANOTRANSFERRIN OR P97) AND GPI AND PY<=2001
L2            11 DUP REM L1 (24 DUPLICATES REMOVED)
L3             9 S (MELANOTRANSFERRIN OR P97) AND (CHONDROCYTE OR CHONDROGENESIS
L4             6 DUP REM L3 (3 DUPLICATES REMOVED)

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=> LOG Y

COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
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FULL ESTIMATED COST                               59.45      59.66

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